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Folate intake of the Dutch population according to newly established liquid chromatography data for foods[1–3]

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ABSTRACT

Background: Determining folate intake is difficult because existing folate data in food-composition tables are scarce and unreliable.

Objective: The purposes of this study were first to analyze 125 of the most important foods that contribute to folate intake in the Netherlands and second to estimate the folate intake of a representative sample of the population.

Design: We analyzed the folate content of foods by using a newly developed HPLC trienzyme method combined with an affinity chromatography cleanup step. These results were then used to estimate the folate intake of persons aged 1–92 y who participated in the second Dutch National Food Consumption Survey (DNFCS) in 1992 (n = 6218).

Results: For 35 important folate-containing foods, the mean relative folate contents measured by HPLC were 66%, 80%, and 77% of values for comparable foods included in the British food-composition table; the Ministry of Agriculture, Fisheries and Food table; and the US Department of Agriculture database, respectively. P values for comparison of relative values with 100% were 0.001, 0.171, and 0.144, respectively. The mean dietary folate intake of the DNFCS participants was 182 ± 119 μg/d. Intake of supplement users (n = 86) was 344 μg/d, with 147 μg/d from supplements. On the basis of these findings, 42% of men and 54% of women do not meet current Dutch recommendations of 60 μg/d for children and 200 μg/d for adults.

Conclusions: Total folate quantities in foods, analyzed by HPLC, are ≈25% lower than amounts listed in recent food-composition tables estimated by use of the microbiological method. On the basis of these new data, ≈50% of a representative Dutch population sample does not meet the current recommendations for folate intake. *Am J Clin Nutr* 2001;73:765–76.

KEY WORDS Folate, folate intake, consumption survey, HPLC, foods, trienzyme treatment, food-composition table, Dutch National Food Consumption Survey

INTRODUCTION

Folates are part of an extended family of polyglutamates (usually containing 5–7 glutamyl residues) of pteroic acid and related analogues that qualitatively exhibit the biological activity of folic acid. It has become clear that folates play important roles not only in the prevention of neural tube defects (1, 2), but possibly also in the etiology of cardiovascular diseases (3, 4) and cancer (5–7).

Average folate intakes from foods for adults, as reported in various European countries, range between 168 and 326 μg/d (8). It has been impossible to compare folate intakes between different countries because of the absence of reliable data for folates in food products. Tamura (9, 10) suggested that all food folate tables be reevaluated to obtain more reliable values.

The total folate content of foods is usually determined by microbiological assay (11, 12). However, these assays have poor precision and fail to differentiate between several folates. Determination of folate monoglutamates is not possible because the microorganisms also respond to di- and triglutamylfolates. The microorganisms might respond unequally to various folate forms; additionally, certain food components could stimulate or inhibit bacterial growth, resulting in unreliable data (13).

The average bioavailability of folates from foods has been estimated at ≈50% (14). The bioavailability of folate monoglutamates might vary between 70% and 120% relative to folic acid (100%) (15). More detailed information about food folate composition is needed to accurately describe intakes and to evaluate the results of bioavailability studies and epidemiologic studies related to disease endpoints.

So far, few studies have provided detailed information on the folate composition of foods (16–21). In addition, the extraction procedure used in these studies was incomplete for certain products because no amylase or protease was used to release folates from cereal or milk products, respectively. Anion-exchange chromatography, an unspecific cleanup step that results in many compounds that interfere with chromatography, is a drawback in most of the methods applied to date. Recently,

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affinity chromatography was used as an improved purification method by Selhub (22) and was applied to food folates by Seyoum and Selhub (23). For the HPLC analysis of folates in cereal-grain products, this cleanup step was used successfully by Pfeiffer et al (24). The analytic methods used in the present study are based on the work of these investigators.

The purpose of our study was 2-fold: first, to analyze the folate content of the most important foods contributing to folate intake, and second, to estimate the folate intake of a representative sample of the Dutch population on the basis of these newly assessed folate values. An improved and validated HPLC method (25) was used to gather more detailed and valid information about food folate composition.

SUBJECTS AND METHODS

Foods for folate analysis were selected on the basis of the second Dutch National Food Consumption Survey (DNFCS), which was carried out in 1992 (26). The first survey was executed in 1987 and is described by Hulshof (27). The DNFCS comprises 6218 noninstitutionalized persons aged 1–92 y in 2475 households selected from a stratified probability sample in the Netherlands. Information on food consumption was obtained with a 2-d dietary record on 2 consecutive days per person, resulting in data for mean consumption of foods in g/d. The survey was distributed equally over the 7 d of the week and over 1 y (except holidays) from January to December 1992. Data on the use of dietary supplements were obtained by means of a semi-open-ended question asking for types, doses per day, and brand names.

Food sample selection

First, the folate intake of all foods mentioned in the DNFCS was calculated by using data from international food tables compiled by Brants and Hulshof (28). Most of these data originated from the British food-composition table (29). Next, 125 food items were selected for chemical analysis (**Appendix A**); these foods contributed ≈90% of total folate intake according to the existing data in the international food tables. The selected food items were mainly vegetables, fruit, bread, milk and milk products, and meat products.

Sample composition

For all foods selected, information on the most important brands sold and on market shares was obtained from commodity boards and a commercial market research agency. The most frequently consumed fruit and vegetable varieties were collected from the major sales channels (eg, marketplaces, supermarkets, and greengrocers). In the Netherlands there are few distribution centers for fruit and vegetables, so samples were collected at local supermarkets, greengrocers, and marketplaces. If folate amounts in foods were suspected to be subject to regional variations, samples were collected from the most important sales channels in 3 regions: Maastricht (south), Gouda (west), and Zwolle (north and east). This was done for milk and milk products and bread. When the season could be a cause of variations in folate amounts, for example, in vegetables, samples were acquired in various seasons. For 3–5 of the main brands of a food item and sales locations for items for which no brand names were used (eg, vegetables, fruit, and bread), 3 different production codes or sales locations were sampled in the units in which the products were sold. Selected brands or sales channels covered >75% of the supply. All samples were purchased in 1996 and 1997. Because folates might be subject to destruction by exposure to heat, air, and light during food preparation, foods were analyzed as consumed to determine the actual intake of folates.

Samples of brands with different production codes or from different sales channels were mixed, by brand or sales channel, and prepared immediately according to normal household practice (30). Subsequently, different brands or sales channel samples were mixed according to the corresponding market shares, yielding the final sample for analysis. All sampling operations were described in a sampling plan (31). Prepared samples (2–3 kg) were homogenized immediately in liquid nitrogen. About 200 g was stored at –80 °C. Analysis usually took place within 1–2 mo of storage. Several vegetable samples that had been analyzed for carotenoids by Goldbohm et al (32) were also used for folate assessment.

Quality control

Several procedures were carried out to validate the means of homogenization, examine moisture changes, and determine analytic quality control. To validate the homogenization procedure, ≈2.5 kg spinach was prepared under normal household practice conditions and was immediately frozen and homogenized in liquid nitrogen. The homogenized sample was divided into 10 polypropylene jars. Each jar was filled with ≈100 g homogenate and stored at –80 °C until analyzed. From every jar, 2 test portions of ≈5 g were taken for folate analysis.

To examine possible moisture changes during the homogenization procedure, 10 samples of vegetables and fruit were homogenized with and without liquid nitrogen. These samples included broccoli, banana, orange, kiwi, red cabbage, potato, spinach, kale, snap beans, and chicory.

Analytic quality control was implemented by use of certified reference materials (CRMs) for folates supplied by the Institute for Reference Materials and Measurement in Geel, Belgium. These materials included lyophilized pig liver (CRM 487), milk powder enriched with folic acid (CRM 421), vegetable mix (CRM 485), and whole-meal flour (CRM 121). CRMs were used as controls in each series of sample analysis.

Analysis

Detailed information about the method of analysis was published previously (25). Briefly, folates from food samples were extracted by homogenization in a Ches-Hepes buffer (pH 7.8; 2% ascorbic acid and 10 mmol 2-mercaptoethanol/L) followed by heat treatment (10 min in a boiling water bath). A first aliquot was analyzed without the addition of any enzymes (treatment 1) to estimate the monoglutamate content of the samples. In a second aliquot, folate concentrations were quantified after the addition of rat plasma conjugase (γ-Glu-X carboxypeptidase; treatment 2) to establish the sum of monoglutamtates and polyglutamates. In a third aliquot, folate concentrations were determined after treatment with rat plasma conjugase plus protease and amylase (treatment 3). The difference between the folate amounts assayed in treatments 1 and 2 represents the folate polyglutamate content. The difference between the folate amounts assayed in treatments 2 and 3 reflects matrix-bound folates. After purification by affinity chromatography, folate monoglutamates were measured by using an HPLC method with fluorescence and diode array detection. All analyses were performed under subdued light. This procedure was used to assess

the most abundant folate forms naturally present in foods, including tetrahydrofolate (H_4folate), 5-methyltetrahydrofolate (5-CH_3-H_4folate), 5-formyltetrahydrofolate (5-CHO-H_4folate), 10-formylfolic acid (10-HCO-folic acid), 10-formyldihydrofolate (10-HCO-H_2folate), and folic acid.

Comparison with other studies and values

Folate contents (by HPLC) in 35 important folate-containing foods such as milk, vegetables, fruit, potatoes, and bread were compared with folate amounts reported in 3 other tables: *McCance and Widdowson's The Composition of Foods* (29), derived from microbiological analysis carried out before 1990; the folate contents reported in 1996 by the British Ministry of Agriculture, Fisheries and Food (MAFF) (33), also based on microbiological analyses; and microbiological data from the US Department of Agriculture (USDA) *Nutrient Database for Standard Reference* (34). The choice of 35 foods for comparison was based on the availability of folate data in all 3 food-composition tables.

Seasonal variations

For vegetables, fruit, potatoes, and milk products, samples were taken 2 or 3 different times over a 1-y period. For each vegetable, the choice of the sampling time was based on the supply and varieties available. The following vegetables were sampled at various times: endive, beets, leeks, lettuce, spinach, tomatoes, carrots, Brussels sprouts, snap beans, and green beans. Potatoes were purchased immediately after harvesting and at the end of the winter season. Analytic results for the various sampling periods were pooled if values were within the margins of reproducibility. Mean relative within-laboratory reproducibility for 5-CH_3-H_4folate in the 4 matrices validated (25) was 30%. Within-laboratory reproducibility was defined as 1.6 × repeatability (35, 36).

The following fruit varieties were sampled in November and February: orange, grapefruit, tangerine, banana, and kiwi. These months were selected for these products on the basis of information from product boards indicating that consumption levels were highest during the first and last quarters of the year. The following milk products were sampled in August (cows in meadow) and in March (cows at stable): whole milk, low-fat milk, skim milk, buttermilk, whole yogurt, low-fat yogurt, and vanilla custard.

Cooking losses

The purpose of the present study was to analyze food products in the form in which they are consumed. Thus, some vegetables that can be consumed either raw or cooked were analyzed as such. First, folates were analyzed in cooked products. Second, the folate contents of cooked products were recalculated to the raw state by correcting for weight loss during preparation. These concentrations were then compared with the folate contents of the raw products, allowing us to calculate folate losses. Cooking losses of folates for endive, spinach, onions, red cabbage, carrots, and cauliflower were examined.

Endogenous conjugases in vegetables and fruit may cleave folate polyglutamates into monoglutamates during preparation, resulting in lower polyglutamate amounts in the cooked products than in the immediately heated products. Endogenous conjugase is usually deactivated during cooking (37). Raw spinach was chopped before storage at –80 °C, resulting in higher monoglutamate contents (Appendix A). To investigate the rate of this deconjugation, raw spinach was chopped and then analyzed for folates after 10, 30, and 60 min of storage at room temperature.

Calculation of intake

To establish a more valid estimate of folate intake for the participants of the DNFCS, the food folate values assessed with use of the new HPLC method were used to recalculate folate intake. The random sample of participants in the DNFCS deviated from the Dutch population at large with respect to sex and age distribution. Young children were overrepresented, leading to an underestimation of the consumption of many food products and of the folate intake in the Dutch population. Although the bias is relatively small, we corrected folate intake for sex and age in calculations based on the total database to obtain a theoretically more accurate estimation. The corrections were used only for calculating the values for the complete database. Because all persons belonging to the same sex-age group have the same correction, calculations in the various groups need not take correction into account (26). Folate contents of those products not included in the above analysis were estimated in several ways. First, analytic values from Appendix A were adopted for comparable foods. For example, the folate content of canned endive was adopted from the analyzed value of fresh, cooked endive. The folate content of low-fat chocolate milk was adopted from the analyzed value of whole chocolate milk because there are small differences in the folate contents of whole and low-fat milks. Second, data from other HPLC methods (19–21) or the MAFF report (33) were selected. Finally, folate amounts in the remaining products were calculated from recipes or estimated to be 27% less than the values listed in the British food-composition table (29).

Folate intake from supplements was calculated by using information on folic acid content collected from product labels and on the number of tablets used per day. All folic acid supplements and multi-vitamin-mineral preparations were included, except if detailed information on nutrient content was missing. Intakes were calculated for men and women separately and by age groups. To investigate whether persons with high food folate intakes also took supplements, food folate intake was divided into tertiles and the number of supplement users in each tertile was counted.

Folate intakes were compared with the current recommendations in the Netherlands, ie, 60 μg/d for children to 200 μg/d for adults; the minimum requirement for adults is assumed to be 100 μg/d and that for pregnant women or women capable of becoming pregnant is 400 μg/d (38, 39). Recently, a new dietary reference intake (DRI) of 400 μg DFE (dietary folate equivalents) was reported in the United States (40). The DFE unit corrects for a greater bioavailability of synthetic folic acid than of natural folates; 100 μg provided as food folate equals 100 μg DFE, whereas 100 μg provided as folic acid equals 170 μg DFE.

Statistical analysis

Quality-control data and folate intake data are presented as means ± SDs. The certified value for 5-CH_3-H_4folate in mixed vegetables (CRM 485) is presented as the mean (95% CI) of the data set averages. Paired Student's *t* tests were performed at the $P < 0.05$ level of significance. To validate the homogenization procedure, folate results for 10 duplicate analyses in spinach were compared by means of a paired Student's *t* test. Between-group and within-group variations were examined with analysis of variance. Moisture contents of fruit and vegetables homogenized with 2 procedures were compared by means of a paired Student's *t* test.

The relative values of the HPLC measurements with respect to results in 3 other food-composition tables were compared with 100% by means of paired Student's *t* tests on the log-transformed

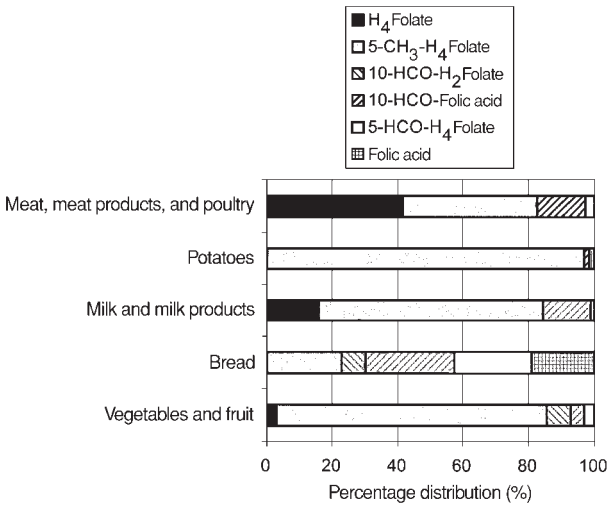

FIGURE 1. Folate vitamer distribution in important product categories. H_4folate, tetrahydrofolate; 5-CH_3-H_4folate, 5-methyltetrahydrofolate; 10-HCO-H_2folate, 10-formyldihydrofolate; 10-HCO-folic acid, 10-formylfolic acid; 5-HCO-H_4folate, 5-formyltetrahydrofolate.

data. For these 3 tests, a Bonferroni-corrected *P* value of 0.017 was used. Log-transformed data were used to improve normal distribution as described by Strike and Dipbiom (41).

RESULTS

Selection of foods for HPLC analysis

A mean folate intake of 251 ± 97 μg/d (median: 234 μg/d) was calculated (42) for a representative sample of the Dutch population with use of the 1992 DNFCS (n = 6218) and data from international food tables compiled by Brants and Hulshof (28). Intake of folic acid from supplements was not included in these calculations. Food items that provided >0.1% of total folate intake were selected for HPLC analysis (Appendix A). Several additional foods were analyzed because of their high folate contents: liver, marmite, wheat germ, and wheat bran. A total of 43 vegetables and fruit, 12 types of bread, 14 milk products, 8 meat products, and 48 other foods were selected for analysis.

Quality control

There were no significant differences in 5-CH_3-H_4folate concentrations among 20 portions of homogenized spinach (CV: 5.6%). Additionally, between-portion variation was not significantly different from within-portion variation, nor was there any significant difference in moisture content between vegetable and fruit samples homogenized in liquid nitrogen and ordinary mixed samples.

Mixed vegetables (CRM 485) was used as a control in almost all of the series analyzed. Results for 5-CH_3-H_4folate in this material were within the confidence limits of the certified HPLC value. The mean concentration measured was 2.10 ± 0.19 μg/g (n = 18), whereas the certified value for this material is 2.14 μg/g (95% CI: 1.72, 2.56). Milk powder (CRM 421) and whole-meal flour (CRM 121) were each also used once. Folate quantities for these materials were within margins of certified values.

Analytic results

The results of the folate analyses in foods are summarized in Appendix A, which shows the amounts of individual folates in each food item as well as the total folate concentration, calculated as folic acid. Folate amounts ranged from 0 to18.27 μg/g for apple juice and marmite, respectively. Percentages of polyglutamates are also presented for each food item. Vegetables and fruit with the highest folate amounts were broad beans, Brussels sprouts, spinach, strawberries, and kiwis, containing, respectively, 1.50, 0.87, 0.83, 0.65, and 0.23 μg folates/g. Other products with high folate concentrations included wheat germ (0.90 μg/g) and chicken liver (13.85 μg/g).

The vitamer distribution for the most important product categories is illustrated in **Figure 1**. 5-CH_3-H_4Folate was the most abundant vitamer in foods. In the food groups of vegetables and fruit, bread, milk products, potatoes, and meat products, 62% of all vitamers were 5-CH_3-H_4folate. Relatively high amounts of H_4folate (the most unstable folate) were found in meat and meat products.

Polyglutamate and matrix-bound folate contents measured after treatments 1, 2, and 3 are listed in **Table 1**. Samples treated with amylase and protease during extraction yielded higher folate concentrations in fruit (16%) and milk products (21%) than did samples not treated with these enzymes. Of the total folates in food, 71% were polyglutamates.

TABLE 1
Polyglutamate and matrix-bound folate contents of various foods and food groups[1]

	Sum of folates	Polyglutamates[2]	Matrix-bound folates[3]
	μg/g	%	%
Potatoes (n = 3)	0.12 ± 0.03	90 ± 2	0
Vegetables (n = 24)	0.31 ± 0.33	80 ± 21	0
Fruit (n = 6)	0.27 ± 0.21	70 ± 13	16 ± 13
Bread (n = 12)	0.26 ± 0.07	66 ± 27	—[4]
Milk and milk products (n = 14)	0.05 ± 0.03	64 ± 21	21 ± 18
Meat, meat products, and poultry (n = 8)	5.22 ± 5.29	57 ± 34	0

[1] $\bar{x}$ ± SD.
[2] Difference between folate amounts assayed after treatment 1 (without the addition of any enzymes) and treatment 2 (after the addition of rat plasma conjugase).
[3] Difference between folate amounts assayed after treatment 2 (after the addition of rat plasma conjugase) and treatment 3 (after the addition of rat plasma conjugase plus protease and amylase).
[4] Not determined.

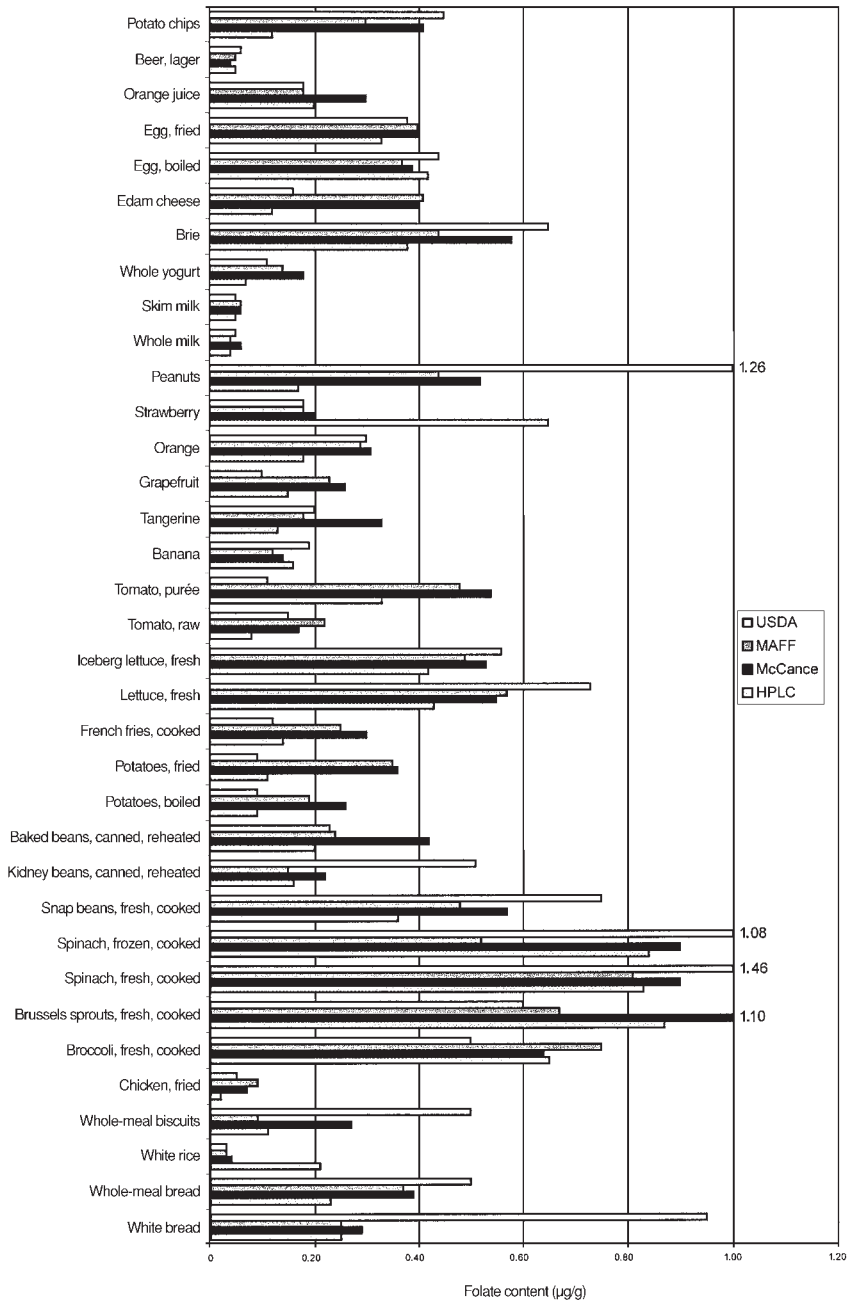

FIGURE 2. The folate contents of 35 foods on the basis of HPLC analysis compared with contents reported in 3 other databases: USDA, US Department of Agriculture *Nutrient Database for Standard Reference* (34); MAFF, British Ministry of Agriculture, Fisheries and Food table (33); McCance, *McCance and Widdowson's The Composition of Foods* (29).

Comparison with other studies and values

Results of comparisons of HPLC-determined folate contents with values listed in 3 other food-composition tables are presented in **Figure 2**. Concentrations determined by HPLC ranged from 0.03 to 0.81 μg/g. The mean relative value of the HPLC results was 66% (95% CI: 54%, 82%) of the values given for comparable foods in McCance and Widdowson's table and was significantly different from 100% ($P = 0.001$ after Bonferroni correction). The mean relative values of the HPLC results with respect to concentrations for comparable foods included in the MAFF table and the USDA nutrient database were 80% (64%, 101%) and 77% (59%, 100%), respectively, and were not significantly different from 100%.

Seasonal variations

Analytic results for various vegetables sampled in different seasons were within margins of laboratory reproducibility values. There were no significant differences in folate intake from these vegetables on the basis of the arithmetic means of results for every season or the means corrected for the number of months by growing season and the percentage of users in several seasons.

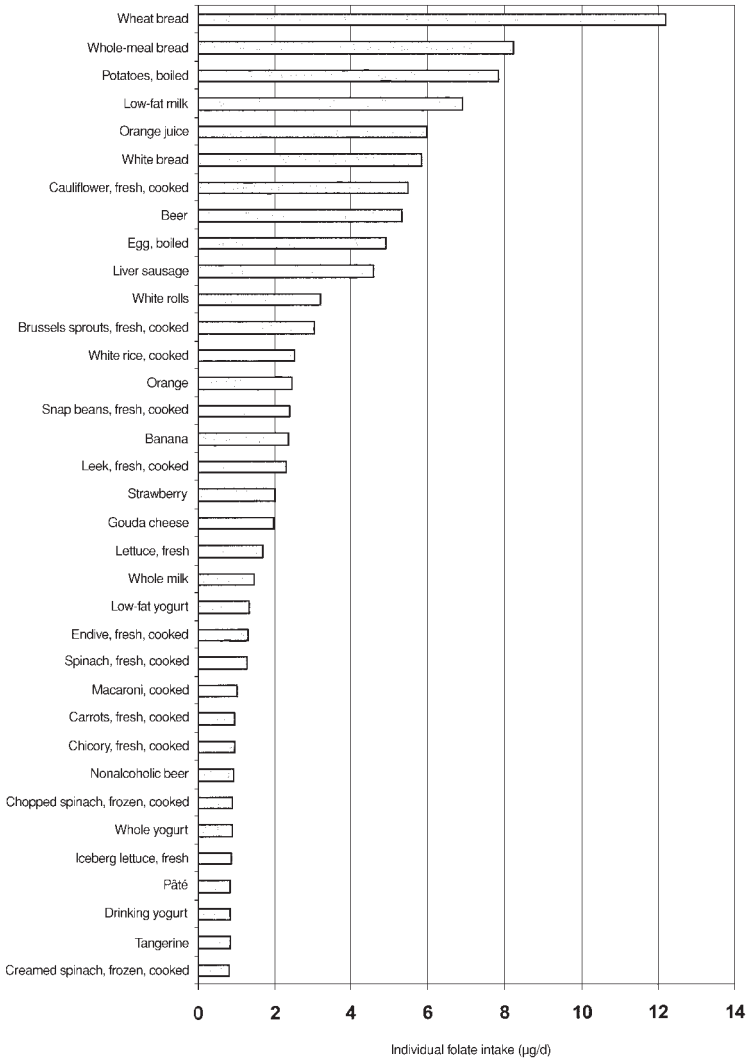

FIGURE 3. The 35 most important food items contributing to folate intake in the Dutch population aged 1–92 y according to the 1992 Dutch National Food Consumption Survey (n = 6218). These 35 products provide ≈60% of total folate intake.

Folate contents of fruit were not significantly different between samples taken in November and those taken in February. Folate concentrations in milk products were also not significantly different when sampled in August or March.

Cooking losses

Cooking losses of folates from endive, spinach, onion, and red cabbage were 56%, 52%, 40%, and 29%, respectively. In cooked carrots and cauliflower, folate contents increased by 15% and 11%, respectively. The percentage of monoglutamates in chopped spinach increased from 25% to 37% to 54% after 10, 30, and 60 min of storage at room temperature, respectively.

Folate intake

We calculated a mean dietary folate intake of 182 ± 119 µg/d (median: 164 µg/d) for a representative sample (aged 1–92 y) of the Netherlands population with use of the 1992 DNFCS (n = 6218) and the newly established HPLC values for folates in foods. Foods sampled and analyzed in the procedures described provided 73% of total folate intake. Vegetables, fruit, and potatoes provided >33% of the daily dietary folate intake (22%, 6%, and 7%, respectively). Bread, milk products, and meat products supplied another ≈33% of the daily dietary folate intake (19%, 9%, and 11%, respectively). Other food products provided 26% of daily folate intake.

Shown in **Figure 3** are the relative contributions to total daily dietary folate intake of 35 of the most important sources of folate among participants in the DNFCS. Wheat bread made the greatest contribution (12.3 µg/d) to folate intake in the Netherlands, followed by whole-meal bread (8.3 µg/d) and potatoes (7.9 µg/d). In **Figure 4**, folate intake is subdivided by sex and age categories. In the whole DNFCS population, total folate intake in men was significantly higher than that in women ($P < 0.05$). For the age categories of 1–4, 4–7, 10–13, 16–19, and >65 y, total folate intake among men and women was not significantly different. Shown in **Table 2** are the mean

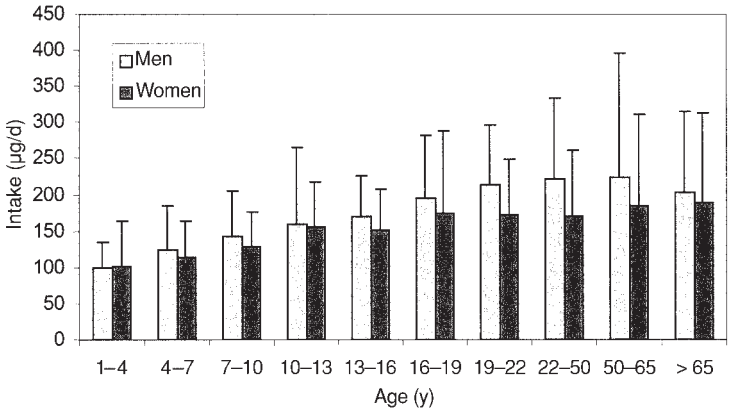

FIGURE 4. Mean (+SD) folate intakes of the Dutch population aged 1–92 y by age and sex in the 1992 Dutch National Food Consumption Survey (n = 6218). Intakes of folic acid as supplements are included.

daily folate intakes of non-supplement-users and supplement users in the DNFCS. Total folate intake was also calculated according to the new DRI standards (40). The mean daily dietary folate intake for participants aged ≥16 y was 193 ± 112 μg/d (n = 4777, 95th percentile: 319 μg/d; men: 215 ± 120 μg/d; women: 173 ± 100 μg/d).

According to our calculations, 42% of men and 54% of women in the DNFCS did not meet the current Dutch folate recommendations of 60 μg/d for children and 200 μg/d for adults. In the DNFCS, 3.6% of adult men and 10.6% of adult women consumed less than the minimum requirement of 100 μg/d. According to the new DRI standards, 97% of the participants in the DNFCS did not meet folate requirements.

The mean folate intake for pregnant women (n = 58; mean age: 30 y) was 187 ± 62 μg/d (range: 54–315 μg/d). None of these women used folic acid supplements. The mean folate intake for women capable of becoming pregnant (16–50 y) was 172 ± 91 μg/d (range: 18–1322 μg/d). The contribution of folic acid from supplements to this intake was marginal (2 μg/d).

Intake from supplements

The total folate intake of the 108 supplement users (1.7% of population) was 324 μg/d, of which 147 μg/d was from vitamin supplements (range: 7.5–800 μg/d; median: 100 μg/d; 95th percentile: 400 μg/d). For 19% of the supplement users, folate intake was still below the current recommendation of 200 μg/d. Of the supplement users, 23% had intakes >400 μg/d. One person had an intake >1000 μg/d. Fifty-eight percent of the supplement consumers were women and 80% of the users were aged ≥16 y. The mean folate intake for supplement users calculated according to the new DRI standards was 427 ± 232 μg DFE/d. For these supplement users, 60% had folate intakes below the DRI of 400 μg DFE/d. When total folate intake without supplements was divided into tertiles, the numbers of supplement users were equally spread across the low, medium, and high categories of food folate intake (37, 36, and 35, respectively).

TABLE 2
Mean daily folate intake and total folate intake calculated according to new dietary reference intake standards for non-supplement-users and supplement users in the 1992 Dutch National Food Consumption Survey[1]

	Non-supplement-users	Supplement users
	μg/d (DFE/d)	*μg/d (DFE/d)*
Population	178 ± 106 (178 ± 106) [6110]	324 ± 148 (427 ± 232) [108]
<16 y	130 ± 64 (130 ± 64) [1419]	248 ± 106 (351 ± 180) [22]
≥16 y	192 ± 112 (192 ± 112) [4691]	344 ± 152 (446 ± 240) [86]

[1] $\bar{x}$ ± SD; n in brackets. DFE, dietary folate equivalents (40).

DISCUSSION

Analytic findings

The present study provides information on the folate composition of a large set of foods eaten regularly in the Netherlands. All aspects of the study complied with the quality-control criteria for the assessment of food folate composition data emphasized by Holden et al (J Holden, G Beecher, R Doherty, et al, unpublished observations, 1997). In the present study, the sample selection and sampling plan for folate analyses were also based on the considerations put forward by Greenfield and Southgate (43). The purpose of our study was to determine the actual intake of folates after food preparation.

Our results showed that the method of homogenizing the samples in liquid nitrogen yielded a homogeneous composite with no significant change in moisture content. Analyses of CRMs showed good repeatability between analytic runs.

For the vegetable extracts, potatoes, and meats, no statistically significant increases in folate amounts were observed after the addition of protease and amylase. For these products, the average analytic results were calculated for the treated and untreated extracts. In contrast, folate concentrations increased by 16% and 21% in the fruit and milk products, respectively, after the addition of protease and amylase. The amount of extra release in bread and pasta was not determined because untreated extracts could not be applied to the affinity chromatography columns. The use of amylase and protease resulted in higher folate contents and agrees with recommendations made in other papers (10, 24, 44, 45).

If microbiological methods had been used, H_4folate might have decomposed during the long incubation times. This could be why folate amounts in liver and liver products in current food tables tend to be low. Recently, Vahteristo et al (19) reported HPLC-measured folate concentrations in liver comparable with

those presented in Appendix A: 7.30–14.70 μg/g in pig and beef livers (not cooked) and substantial amounts (4.80–10.50 μg/g) of tetrahydrofolic acid.

Another remarkable finding was the presence of 10-HCO-H_2folate in some vegetables. In some leafy green vegetables (spinach, endive), the ratio between 10-HCO-H_2folate and 5-CH_3-H_4folate was 1:1. 10-HCO-H_2Folate, like 10-HCO-folic acid, is an oxidation product of 10-HCO-H_4folate. These vitamers may have been produced during the preparation or processing of these vegetables. It is unlikely that conversions took place during analysis, in view of the good recoveries of the different folate derivatives. Pfeiffer et al (24) were the first to report the presence of 10-HCO-H_2folate in bread. This vitamer was also found in the present study and was probably introduced by yeast because no 10-HCO-H_2folate is found in whole-meal flour (25). Pfeiffer et al (24) measured folates in unfortified white bread, wheat bread, and white rice by HPLC and affinity chromatography (white bread: 0.21 μg/g; wheat bread: 0.30 μg/g; white rice: 0.14 μg/g) and their findings correspond well with ours (0.25, 0.27, and 0.21 μg/g, respectively). Folate amounts in white rolls were higher than in white bread, probably because twice as much yeast is used in the former.

Seyoum and Selhub (23) described good agreement between the results of HPLC with affinity chromatography and microbiological methods, but analyzed only 10 food products. Less than 4 products were comparable with the foods analyzed in the present study and the folate contents of these items are not listed in their paper so cannot be compared with our results. Several of the HPLC measurements made in the present study agree well with microbiological results listed in one or more of the food tables used for comparison. However, in general, most HPLC-measured folate contents were lower than values found by microbiological assay. Because the most abundant folate vitamers present in food products were determined by HPLC, this finding suggests that nonfolate compounds influence the bacterial growth response, resulting in higher folate contents with the microbiological assay (11). The mean of 35 relative folate values in McCance and Widdowson's food-composition table (29) was 121% of values in the MAFF table (33) and was significantly different from 100% ($P < 0.001$). Both values were obtained with use of the microbiological assay (46) for the same British food products. This indicates a large variation in microbiological results, which could explain part of the difference between our HPLC results and the microbiologically-derived values given in current food tables.

Our HPLC results are ≈27% lower than the folate amounts listed in the British food tables and 23% (NS) lower than the folate amounts described in the USDA nutrient database. This contradicts statements that the folate content in current food tables is underestimated (10).

Seasonal variations in the folate contents of different varieties of vegetables, fruit, potatoes, and milk products were small, as also reported by Vahteristo et al (21) and Mullin et al (47) for some vegetable varieties. Cooking may have released additional folates in carrots and cauliflower, but these differences can probably be explained by a reproducibility value of 30% (25). DeSouza and Eitenmiller (48) reported comparable folate losses for blanched spinach.

Folate intake

With use of our HPLC data, we calculated the dietary folate intake of a representative sample of the Dutch population to be 182 μg/d; in contrast, with use of data provided mainly by the British food table, we calculated the intake of this population to be 251 μg/d. Compared with data for other European countries [291 μg/d for adult men and 247 μg/d for adult women (8)] and the United States [242 μg/d for adults (49) and 283 μg/d for persons aged >6 y (40)], the calculated dietary intake of the Dutch population according to our HPLC data is low. But, as evaluated previously, earlier analytic methods appear to overestimate folate intake, resulting in ≈25% higher folate values.

According to our calculations, which were based on a 2-d dietary record included in a food consumption survey, about one-half of the DNFCS population failed to meet the Dutch folate requirements. Almost no one had a folate intake meeting the new DRI standards. Until recently, foods in the Netherlands were not allowed to be fortified with folic acid. An intake of 200 g vegetables and 2 pieces of fruit daily is currently recommended to enhance the intake of folate and other nutrients. This is approximately double the amount of vegetables and fruit found to be consumed in the 1992 DNFCS. Even if average intakes of vegetables and fruit were doubled (by adding a mean folate intake from vegetables and fruit of 50 μg/d per person), 33% of adults would still not meet the required 200 μg/d. Since regulations requiring the fortification of certain cereal-grain products with synthetic folic acid became effective in the United States in early 1998, new estimated folate intakes for the US population compare favorably with the new DRI standards (50). However, these estimations are not based on actual measurements.

An intake of 100 μg folate/d is deemed to be sufficient to maintain adequate serum folate concentrations in ≥80% of the population (38). Brussaard et al (51) found that among 444 Dutch adults, ≈10% of the men and 10% of the women had low serum folate concentrations on the basis of a cutoff of 7 nmol/L. According to Herbert (52), this concentration indicates early negative folate balance. Our calculations show more or less comparable percentages of men and women with intakes below the minimum requirement of 100 μg/d. Thus, the folate intake calculations in the DNFCS appear to be confirmed by the serum folate concentrations found in Brussaard et al's study.

A folic acid intake of 400 μg/d is advised to prevent neural tube defects. A dietary folate intake of 350 μg/d is recommended to maintain low homocysteine concentrations in plasma, which might prevent cardiovascular diseases (8). According to our calculations, almost the entire DNFCS population failed to meet these folate intakes.

Of the 15 major contributors to folate intake according to the DNFCS, 6 are also among the 15 most important contributors in the US second National Health and Nutrition Examination Survey (1988–1994) (49). These include whole-meal bread, low-fat milk, white bread, orange juice, beer, and eggs. The other 9 items differed, reflecting differences in dietary patterns. Many foods with high folate contents, such as broad beans, spinach, and broccoli (Appendix A) do not rank high in actual dietary folate intake because of their low consumption rate. Increased consumption of these products could be recommended to enhance folate intake.

Among supplement users, the mean folic acid intake from supplements was ≈50% of total folate intake. The effect of supplement use on total folate intake for the whole DNFCS population was small, however, because only 1.7% of the study population took supplements. In the United States, 28% of the participants in the second National Health and Nutrition Examination Survey reported the use of supplements containing folic acid. In that survey, supplemental folic acid contributed 68% of total folate intake (49). Because the number of supplement users in the present study was distributed evenly in the low, medium, and high food

folate intake groups, we found no indication that only persons with high food folate intakes also took supplements.

In summary, this study provides information on food folate composition with use of a newly developed HPLC method. Folate intake values calculated for a representative sample of the Dutch population with use of these data show a relatively low folate intake compared with the new DRI standards.

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APPENDIX A

Folate content of foods in the Netherlands, 1997–1998[1]

	H_4Folate	5-CH_3-H_4Folate	10-HCO-H_2Folate	10-HCO-Folic acid	5-HCO-H_4Folate	Folic acid	Total as folic acid	Polyglutamates
	μg/g	*μg/g*	*μg/g*	*μg/g*	*μg/g*	*μg/g*	*μg/g*	*%*
Potatoes								
French fries, cooked (*n* = 3)	—[2]	0.14 ± 0.03[3]	—	0.01 ± 0.01	—	0.01 ± 0.01	0.14 ± 0.05	91
Potatoes, boiled (*n* = 10)	—	0.10 ± 0.02	—	—	—	—	0.09 ± 0.02	87
Potatoes, fried (*n* = 10)	—	0.11 ± 0.03	—	—	—	—	0.11 ± 0.03	91
Bread								
Wheat bread (*n* = 1)	—	0.06	0.05	0.06	0.09	0.03	0.27	57
Wheat-malt bread (*n* = 1)	—	0.05	—	0.06	0.05	0.04	0.19	68
White bread (*n* = 1)	—	0.05	0.04	0.05	0.04	0.07	0.25	62
Milk white bread (*n* = 1)	—	0.06	0.04	0.04	0.03	0.02	0.17	88
Whole-meal bread (*n* = 1)	—	0.04	—	0.08	0.10	0.01	0.23	79
Wheat-rye bread (*n* = 1)	—	0.06	—	0.09	0.12	0.02	0.27	80
White rolls (*n* = 2)	—	0.19 ± 0.01	0.07 ± 0.03	0.06 ± 0	0.08 ± 0.04	0.02 ± 0.01	0.39 ± 0.02	89
Croissants (*n* = 1)	—	0.12	0.05	0.05	0.07	—	0.28	85
Currant-raisin bread (*n* = 1)	—	0.02	—	0.10	0.05	—	0.16	99
Rye bread, dark (*n* = 1)	—	—	—	0.05	0.02	0.17	0.23	10
Rye bread, light (*n* = 1)	—	—	—	0.07	0.01	0.10	0.18	20
Swedish crisp bread (*n* = 1)	—	0.13	—	0.05	0.10	0.05	0.31	57
Alcoholic drinks								
Beer, lager (*n* = 2)	—	0.02 ± 0	—	0.04 ± 0	—	—	0.05 ± 0.01	11
Nonalcoholic drinks								
Tomato-vegetable juice (*n* = 2)	—	0.14 ± 0.01	—	—	0.01 ± 0.01	0.03 ± 0.01	0.18 ± 0.01	70
Orange juice (*n* = 8)	—	0.18 ± 0.01	—	—	—	0.02 ± 0.01	0.20 ± 0.02	61
Double-mix fruit juice (*n* = 2)	—	0.06 ± 0	—	—	—	—	0.06 ± 0	47
Beer, lager (*n* = 2)	—	0.03 ± 0	—	0.02 ± 0	—	—	0.05 ± 0	9
Apple juice (*n* = 2)	—	—	—	—	—	—	0 ± 0	—
Eggs								
Egg, boiled (*n* = 2)	—	0.39 ± 0.02	—	0.05 ± 0	—	—	0.42 ± 0.02	2
Egg, fried (*n* = 2)	—	0.32 ± 0.02	—	0.03 ± 0	—	—	0.33 ± 0.02	0
Fruit								
Orange (*n* = 3)	0.01 ± 0.01	0.18 ± 0.01	—	—	—	—	0.18 ± 0.01	73
Grapefruit (*n* = 2)	0.01 ± 0	0.15 ± 0.06	—	—	—	—	0.15 ± 0.06	58
Tangerine (*n* = 2)	0.01 ± 0.01	0.13 ± 0.01	—	—	—	—	0.13 ± 0	57
Banana (*n* = 2)	0.02 ± 0	0.14 ± 0.05	—	0.01 ± 0	—	—	0.16 ± 0.05	85
Kiwi (*n* = 4)	0.01 ± 0	0.23 ± 0.04	—	—	—	—	0.23 ± 0.04	83
Strawberry (*n* = 3)	0.04 ± 0.02	0.49 ± 0.04	—	—	0.14 ± 0.10	—	0.65 ± 0.14	62
Pastry and cake								
Cream cake (*n* = 1)	—	0.03	—	0.01	0.03	0.06	0.13	97
Fruit pie (*n* = 1)	—	—	—	0.02	0.02	—	0.05	0
Gingerbread (*n* = 1)	—	0.01	—	—	—	0.06	0.08	100

(Continued)

APPENDIX A (*Continued*)

Almond-paste pastry (n = 1)	—	0.01	—	0.03	—	—	0.04	35
Whole-meal biscuits (n = 1)	—	0.03	—	0.05	0.04	—	0.11	49
Cereal products								
Wheat germ (n = 3)	—	0.25 ± 0.09	—	0.13 ± 0.03	0.41 ± 0.04	0.16 ± 0.04	0.90 ± 0.14	91
Wheat bran (n = 3)	—	0.02 ± 0.01	—	0.12 ± 0.02	0.31 ± 0.16	0.05 ± 0.03	0.47 ± 0.21	64
Oatmeal porridge (n = 2)	—	0.07 ± 0.01	—	0.01 ± 0	—	—	0.08 ± 0.02	48
Macaroni, cooked (n = 1)	—	0.05	—	0.01	—	—	0.06	0
White rice, cooked (n = 1)	—	—	—	—	0.22	—	0.21	97
Brown rice, cooked (n = 2)	—	—	—	0.01 ± 0	—	0.03 ± 0.01	0.05 ± 0.02	40
Crunchy muesli (n = 1)	—	0.03	—	0.03	0.04	—	0.09	71
Vegetables								
Endive, fresh, cooked (n = 3)	—	0.17 ± 0.07	0.15 ± 0.03	0.05 ± 0.02	0.08 ± 0.09	—	0.42 ± 0.18	51
Endive, raw (n = 1)	—	0.29	—	0.23	—	—	0.50	67
Snap beans, fresh, cooked (n = 3)	—	0.27 ± 0.05	0.06 ± 0.08	0.01 ± 0	0.04 ± 0.06	—	0.36 ± 0.15	100
Snap beans, frozen, cooked (n = 1)	—	0.31	0.09	—	0.03	—	0.41	100
Broccoli, fresh, cooked (n = 6)	—	0.49 ± 0.11	0.13 ± 0.08	—	0.06 ± 0.09	—	0.65 ± 0.24	92
Kale, frozen, cooked (n = 1)	—	0.27	0.16	0.03	0.09	—	0.52	99
Kale, fresh, cooked (n = 2)	—	0.49 ± 0.06	—	0.03 ± 0.03	—	—	0.50 ± 0.08	88
Broad beans, fresh, cooked (n = 2)	—	1.20 ± 0.02	—	0.01 ± 0	0.36 ± 0.09	—	1.50 ± 0.07	86
Spinach, fresh, cooked (n = 2)	—	0.45 ± 0.14	0.25 ± 0.02	0.04 ± 0.03	0.13 ± 0.07	—	0.83 ± 0.06	90
Spinach, raw (n = 1)	—	0.46	0.48	0.11	—	—	1.00	33
Creamed spinach, frozen, cooked (n = 1)	—	0.32	0.39	0.03	—	—	0.70	86
Spinach, chopped, frozen, cooked (n = 1)	—	0.52	0.28	0.06	0.02	—	0.84	80
Cauliflower, fresh, cooked (n = 3)	—	0.57 ± 0.02	—	—	—	—	0.55 ± 0.03	98
Cauliflower, raw (n = 1)	—	0.18	0.17	0.12	—	—	0.44	91
Carrots, fresh, cooked (n = 18)	—	0.16 ± 0.07	—	—	—	—	0.16 ± 0.07	96
Carrots, raw (n = 6)	0.01 ± 0	0.11 ± 0.03	—	0.01 ± 0.01	—	—	0.13 ± 0.03	66
Chicory, fresh, cooked (n = 4)	—	0.19 ± 0.04	—	0.01 ± 0	—	—	0.19 ± 0.04	62
Chicory, raw (n = 2)	—	0.22 ± 0.01	—	0.02 ± 0	—	—	0.23 ± 0.01	87
Red cabbage, fresh, cooked (n = 6)	—	0.22 ± 0.03	—	—	—	—	0.21 ± 0.03	93
Red cabbage, raw (n = 2)	—	0.25 ± 0.03	—	—	—	—	0.24 ± 0.03	75
Red cabbage, preserved, cooked (n = 2)	—	0.14 ± 0	—	—	—	—	0.13 ± 0	92
Red cabbage and apple, frozen, cooked (n = 2)	—	0.17 ± 0.01	—	0.01 ± 0	—	—	0.17 ± 0.01	91
Tomato, raw (n = 4)	0.02 ± 0	0.06 ± 0.03	—	—	—	—	0.08 ± 0.03	74
Grean bean, fresh, cooked (n = 4)	—	0.22 ± 0.02	—	0.01 ± 0	—	—	0.22 ± 0.03	99
Lettuce, fresh (n = 5)	0.02 ± 0.01	0.34 ± 0.11	—	0.08 ± 0.02	—	—	0.43 ± 0.11	82
Onion, raw (n = 2)	—	0.10 ± 0	—	—	—	—	0.10 ± 0	74
Onion, fresh, boiled (n = 2)	—	0.10 ± 0.01	—	—	—	—	0.09 ± 0.01	94
Onion, fresh, fried (n = 2)	—	0.10 ± 0.06	—	—	—	—	0.09 ± 0.05	85
Sauerkraut, cooked (n = 2)	—	0.07 ± 0.01	—	—	—	—	0.07 ± 0	4
Asparagus, fresh, cooked (n = 2)	—	0.58 ± 0.01	—	—	—	—	0.56 ± 0	93
Iceberg lettuce, fresh (n = 3)	0.04 ± 0.01	0.38 ± 0.02	—	0.01 ± 0.01	—	—	0.42 ± 0.03	74
Cucumber, fresh (n = 4)	0.02 ± 0	0.03 ± 0.01	—	—	—	—	0.05 ± 0.01	83
Beet, fresh, cooked (n = 4)	—	0.24 ± 0.12	—	—	—	—	0.23 ± 0.11	46
Beet, raw (n = 2)	—	0.19 ± 0.05	—	—	—	—	0.19 ± 0.05	68
Brussels sprouts, fresh, cooked (n = 6)	—	0.76 ± 0.28	—	—	0.14 ± 0.06	—	0.87 ± 0.28	96
Leek, fresh, cooked (n = 4)	—	0.61 ± 0.30	—	—	—	—	0.58 ± 0.29	95
Tomato purée (n = 2)	—	0.34 ± 0	—	—	—	—	0.33 ± 0	63
Savory sandwich spread								
Peanut butter (n = 1)	—	0.03	—	0.08	0.21	—	0.29	89
Marmite (n = 2)	—	1.45 ± 0.17	1.12 ± 0.10	1.46 ± 0.25	1.23 ± 0.06	13.30 ± 2.18	18.27 ± 2.73	7
Cheese								
Maaslander, 48% fat (n = 1)	—	0.01	—	—	0.07	—	0.07	91
Gouda, 48% fat (n = 1)	—	0.03	—	—	0.08	—	0.10	82
Edam, 40% fat (n = 1)	—	—	—	—	0.11	—	0.12	91
Brie (n = 1)	—	0.10	—	—	0.07	—	0.38	90
Nuts and snacks								
Potato chips (n = 1)	0.05	0.06	—	—	0.01	—	0.12	100
Almonds and raisins (n = 1)	—	0.10	—	—	0.02	0.88	1.04	0
Cocktail snacks (n = 1)	—	0.11	—	—	0.01	—	0.15	82
Peanuts (n = 1)	—	0.05	—	—	0.13	—	0.17	43
Coated peanuts (n = 1)	—	0.06	—	—	0.06	—	0.12	69

(Continued)

APPENDIX A (*Continued*)

Legumes								
Haricot beans, canned, reheated (n = 2)	—	0.15 ± 0	—	0.02 ± 0	—	—	0.17 ± 1	100
Kidney beans, canned, reheated (n = 2)	—	0.13 ± 0	—	0.03 ± 0	—	—	0.16 ± 0	100
Baked beans, canned, reheated (n = 2)	—	0.17 ± 0.01	—	0.02 ± 0	—	0.02 ± 0	0.20 ± 0	75
Complete dishes								
Russian salad (n = 1)	—	0.01	—	0.02	0.07	—	0.09	95
Pizza with meat, heated (n = 1)	—	0.12	0.04	0.02	—	—	0.17	79
Fried rice and egg, Chinese style (n = 1)	—	0.02	—	0.02	—	0.01	0.05	45
Chinese noodles, cooked (n = 1)	—	0.04	—	0.02	—	—	0.06	60
Pancakes (n = 2)	—	0.04 ± 0	—	0.02 ± 0	—	0.01 ± 0.01	0.06 ± 0.02	36
Soup								
Pea soup with meat, homemade (n = 1)	—	0.09	—	0.05	—	—	0.13	93
Vegetable soup, package, cooked (n = 2)	—	0.01 ± 0	—	—	—	—	0.01 ± 0	89
Vegetable soup, canned, heated (n = 2)	—	0.02 ± 0	—	0.01 ± 0	—	0.02 ± 0	0.05 ± 0	51
Candy, sweets								
Chocolate with nuts (n = 1)	—	0.05	—	0.02	0.05	—	0.12	65
Sauce								
Tomato sauce (n = 2)	—	0.13 ± 0.01	—	—	—	0.02 ± 0	0.15 ± 0.02	68
Fish								
Filet of haddock, fried (n = 1)	—	0.03	—	0.01	—	—	0.04	36
Milk and milk products								
Whole milk (n = 2)	—	0.04 ± 0	—	—	—	—	0.04 ± 0	42
Low-fat milk (n = 2)	—	0.06 ± 0.02	—	—	—	—	0.05 ± 0.02	53
Skim milk (n = 2)	—	0.05 ± 0.01	—	—	—	—	0.05 ± 0.01	59
Buttermilk (n = 2)	—	0.02 ± 0.01	—	—	—	—	0.02 ± 0.01	60
Whole yogurt (n = 2)	0.05 ± 0.01	0.02 ± 0.01	—	—	—	—	0.07 ± 0.02	72
Low-fat yogurt (n = 2)	0.04 ± 0	0.02 ± 0	—	—	—	—	0.06 ± 0	100
Low-fat yogurt and fruit (n = 2)	0.04 ± 0	0.03 ± 0	—	—	—	—	0.07 ± 0.02	92
Vanilla custard (n = 2)	—	0.01 ± 0	—	—	—	—	0.01 ± 0	49
Whole chocolate milk (n = 1)	—	0.03	—	—	—	—	0.03	49
Drinking yogurt (n = 1)	0.01	0	—	0.03	—	—	0.04	39
Skim soft-curd cheese (n = 1)	—	0.02	—	0.08	—	0.02	0.11	96
Low-fat soft-curd cheese and fruit (n = 1)	—	0.03	—	0.03	—	—	0.05	79
Whole evaporated milk (n = 1)	—	0.05	—	—	—	—	0.05	63
Low-fat evaporated milk (n = 1)	—	0.05	—	—	—	—	0.05	40
Meat								
Liver sausage (n = 2)	1.76 ± 0.11	0.13 ± 0.03	—	0.01 ± 0.01	0.21 ± 0.02	—	2.07 ± 0.05	29
Pâté (n = 2)	0.76	0.22 ± 0.05	—	0.54 ± 0.17	—	—	1.47 ± 0.40	45
Hamburger, fried (n = 1)	—	—	—	0.02	—	—	2	100
Chicken, fried (n = 1)	0.01	0.01	—	—	—	—	2	97
Pork liver, fried (n = 2)	1.13 ± 0.41	4.08 ± 0.10	—	—	0.37 ± 0.11	—	5.40 ± 0.70	78
Beef liver, fried (n = 2)	7.84 ± 0.24	2.90 ± 0.26	—	—	—	—	10.57 ± 0.02	2
Calf liver, fried (n = 1)	3.08	4.39	—	—	—	—	7.29	38
Chicken liver, fried (n = 1)	1.91	11.65	—	—	0.78	—	13.85	65

[1] H_4Folate, tetrahydrofolate; 5-CH_3-H_4folate, 5-methyltetrahydrofolate; 10-HCO-H_2folate, 10-formyldihydrofolate; 10-HCO-folic acid, 10-formylfolic acid; 5-HCO-H_4folate, 5-formyltetrahydrofolate.

[2] Folate content equals zero.

[3] $\bar{x}$ ± SD.